A MICROSCOPIC METHOD FOR ANAEROBIC CULTIVATION

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The method proposed in this paper is a modification of the usual moist chamber preparation; the anaerobic system is obtained by the absorption of oxygen by alkaline pyrogallate.

APPARATUS

The apparatus required is simple. As may be seen from fig. 1, it consists of a moist chamber containing a cell of H_2O surrounded by a moat of alkaline pyrogallate. The preparation of the anaerobic moist chamber demands no special apparatus other than that usually found in the routine equipment of the laboratory. The large object slide, s, and large outer chamber ring, r_1 (2.5x1 cm.); large coverglass, c, are the same as those used in Hansen's method of single cell cultivation of yeasts. The inner ring, r_2, is not only smaller in diameter (1.5 cm.), but also less in height (0.8 cm.) than the outer ring. If rings of the proper height (0.8 cm.) are not at hand, they may be prepared by grinding down higher rings of the same diameter, such as the 1.5x1 cm. rings usually furnished for routine moist chamber studies.

The chemicals required are pyrogallol, 5% KOH, high melting point paraffin, vaseline or stopcock grease.

MANIPULATION

(a) The preparation of the anaerobic moist chamber is simple and involves little more than the careful sealing of the two rings to the object slides in the relative position shown in the figure.

Thorough sealing of the rings is required. It was found that the following procedure gives good results: A ring of melted paraffin is applied to the object slide by means of a brush or a match in such a way as to give a film or shallow layer of paraffin over both the area covered by the bottom of both rings and the area between them. The area circumscribed by the inner ring is kept free of paraffin. The layer of paraffin is sealed more firmly to the slide by passing a hot spatula or other instrument over the paraffin after its application. Then the small ring is heated slightly, dipped into melted paraffin and sealed to

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the slide by pressing it down on the inner edge of the paraffin layer. A film of paraffin is coated over the outer side of the ring after it is sealed to the slide. Applying an excess of paraffin several times and passing a hot spatula close around both edges of the bottom of the ring after it is fastened to the slide will insure a perfect seal. The large ring is sealed to the slide in the same manner. A film of paraffin is then applied around the inner side of the large ring.

The application of the films of paraffin on the sides of the rings, together with the resealing around the edges of both rings, forms a paraffin-lined moat between the cells. If the moat is not already completely lined with paraffin, this may be accomplished by the introduction of melted paraffin into the moat by means of a capillary pipet. The paraffin lining thus obtained is desirable to obtain the perfect fastening of the rings to the slide by protecting the glass from etching by the alkali. The efficiency of sealing of the rings may be tested by careful examination or by introduction of water into the cells.

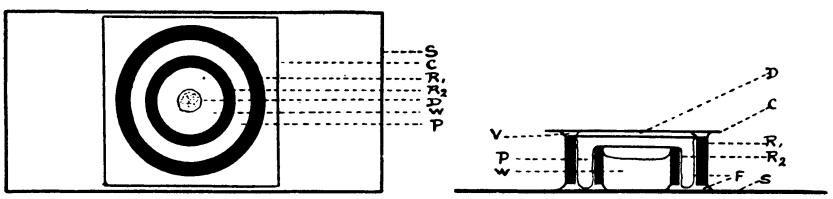

Fig. 1.—Apparatus for anaerobic cultivation (natural size). A, top view; B, cross section; S, slide; C, cover glass; R_1, ring; R_2, ring; D, drop culture; W, water; P, alkaline pyrogallic acid; V, vaseline; F, paraffin.

The most careful preparation of the chambers does not require 15 minutes of the worker's time. They may be used immediately, or may be prepared some time before the occasion. It is usually desirable, however, to prepare them some time before, to permit testing of the sealing.

(b) Preparation of the Anaerobic Culture: Preparation of the anaerobic moist chamber culture involves little more manipulation than that required in the preparation of the usual aerobic moist chamber culture. By means of a small spatula or folded paper, approximately 0.07 gm. pyrogallol is put into the bottom of the paraffin-lined moat. The inner cell is filled with water to a short distance from the top of the ring. The upper edge of the outer ring is then covered with stopcock grease or vaseline.

A drop of recently boiled culture medium is placed on the large cover slip held in Carnet forceps. The drop is inoculated with the anaerobe under investigation; the cover slip is then inverted. At this time 5% KOH is introduced into the moat; the inoculated cover slip is then sealed on the top of the large ring.

(c) Incubation and Observation: The anaerobic moist chamber culture so prepared is now ready for microscopic observation and incubation. The culture can be incubated on the microscope stage and be under the direct observation of the investigator throughout the history of the culture.

Anaerobic hanging block preparations could be prepared with practically the same technic.

DISCUSSION OF THE METHOD

Anaerobic conditions are obtained by the absorption of oxygen by the alkaline pyrogallate solution in the moat surrounding the cell of water. The amount of pyrogallol used presents an excess over that required to absorb the small quantity of oxygen present in the part of the chamber not occupied by the pyrogallate and water. The total volume of the chamber is approximately 5 c c, but the prepared chamber contains less than 2 c c free air space. These relations insure low oxygen concentrations in the prepared chamber during incubation.

The chamber moat presents approximately 3 square cm. surface area for oxygen absorption by the pyrogallate. The large surface exposure gives a rapid absorption of oxygen.

Observation of the culture is made by means of the light passing through the inner cell of water as in the case of the usual moist chamber preparation. Loosening of the inner ring, which may result in entrance of the dark pyrogallate solution into the inner cell, will interfere with the illumination of the object. This will occur in poorly prepared chambers.

Evaporation and change in concentration of the culture medium is reduced to a minimum by the saturation of the chamber with water vapor from the inner cell, which presents a large surface of water.

This anaerobic moist chamber was used successfully to study the growth and development of obligate anaerobes, namely B. tetani and B. botulinus. The photomicrograph of the culture could be made directly at different intervals. At the same time, it was demonstrated that the growth of an obligate aerobe, viz., B. subtilis, in the chamber, was inhibited.

SUMMARY

By the use of this anaerobic moist chamber, the growth and development of anaerobic micro-organisms may be studied by direct microscopic observation throughout the uninterrupted history of the culture.

This microscopic method of anaerobic cultivation possesses the general advantages of oxygen absorption anaerobic methods.

In addition, it possesses the following advantages over the older microscopic oxygen absorption anaerobic methods: simplicity of apparatus, ease of manipulation, good illumination of object, high degree of oxygen absorption, rapid decrease in oxygen concentration, and minimum evaporation and change in concentration of the culture medium.